

Ganoderma gibbosum newly recorded from Pakistan

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ABSTRACT—*Ganoderma gibbosum* is a new record for Pakistan, identified by morphological and molecular data. DNA sequence analysis of ribosomal 5.8S rRNA gene including the flanking nuclear ribosomal DNA-ITS regions confirmed the Pakistani specimens as *G. gibbosum*. Morphologically, the specimens are characterized by their non-laccate sessile basidiomata, truncate, short fine minute inter walled pillars, elongated basidiospores ($7.5\text{--}10.2 \times 4.6\text{--}5.9\ \mu\text{m}$), and greyish to greyish-brown dull pileal surface.

KEY WORDS—*Elfvingia*, *Ganodermataceae*, morphology, polypore, taxonomy

Introduction

Imazeki (1952) divided *Ganoderma* into two subgenera: *G. subg. Ganoderma* [typified by *G. lucidum* (Curtis) P. Karst.] and *G. subg. Elfvingia* (P. Karst.) Imazeki [typified by *G. applanatum* (Pers.) Pat.] (Gottlieb & Wright 1999a,b). Species of *G. subg. Ganoderma* are laccate, with palisade and inflated hyphal ends (Zhao & Zhang 2000), whereas, species of *G. subg. Elfvingia* are non-laccate, with palisade absent and a dull basidiome (Smith & Sivasithamparam 2000; Richter & al. 2015).

A few species reported from Pakistan in *G. subg. Elfvingia* are *G. australe* (Fr.) Pat., *G. tornatum* (Pers.) Bres., and *G. lipsiense* (Batsch) G.F. Atk. (Li & al. 2010). Taxonomic studies regarding *G. subg. Elfvingia* rely on macro- and micromorphological characters to identify and delineate the species. However,

morphological concepts are insufficient to correctly define *Ganoderma* specimens (Moncalvo & Ryvarden 1997), as influenced by environmental conditions and geographic origin (Torres-Torres & Guzmán-Dávalos 2012). The use of the “biological” and “phylogenetic” species recognition concept has significantly contributed in genus delimitation and species identification (Moncalvo & al. 1995; Zhou & al. 2015).

Such studies are still restricted due to limited numbers of specimens, geographic regions, validated material, and misidentifications; and the public database records constitute major complications that are obstacles preventing the drawing of robust phylogenetic conclusions (Fryssouli & al. 2020). Evolutionary relationships among phylogenetically related taxa from different continents are quite imperfectly known, with very important gaps revealed in geographical distributions as well (Welti & Courtecuisse 2010). ITS data might be helpful in resolving the taxonomy of the *G. gibbosum* complex, and comprehensive studies are necessary for any taxonomic decision (Moncalvo 2000).

This study aims to prove that the specimens from Pakistan used in this study were correctly determined as *Ganoderma gibbosum*, through detailed observations of both morphological and molecular characters.

Materials & methods

Studied materials

Specimens were collected in the 2018–19 monsoon season from Changa Manga Forest, District Kasur, Punjab, and from University of the Punjab, New Campus, Lahore, Pakistan. Both sites are covered by *Dalbergia sissoo* Roxb. ex DC. and *Acacia nilotica* (L.) Willd. ex Delile. The average annual rainfall is 1232 mm and the temperature 24°C (Ahmad & al. 2014). Size, shape, and color of basidiomata were noted. The color descriptions followed Munsell (1975). Microscopic structures were observed from cross sections of the basidiome after soaking in KOH (5% w/v), stained with Congo red (1%) and viewed under a MX4300H compound light microscope (Meiji Techo Co., Ltd., Japan). The data of micromorphological features were recorded from at least 30 measurements of face view and side view at a magnification of 100X. Basidiospore measurements are presented as length × width (Nagy & al. 2010). Morphological descriptions of the microscopic features use (in part) the terminology defined by Torres-Torres & Guzmán-Dávalos (2012). The specimens were deposited in Mycology Herbarium, Laboratory of Mycology, Institute of Botany, Academy of Sciences of the Republic of Uzbekistan, Tashkent 100125, Uzbekistan (TASM-ZSH).

DNA extraction, amplification, and sequencing

Modified CTAB procedure was followed to extract total genomic DNA from the dried specimens (Doyle & Doyle 1987). ITS1+5.8S+ITS2 rDNA region (henceforth referred to as the ITS region) was used to study the target specimens. This region was amplified by using primers ITS1 and ITS2 (White & al. 1990). Reaction mixtures (20 µl) contained 0.5 µl template DNA, 8.5 ml distilled water, 0.5 µl of each primer, and 10 ml PCR mix [DreamTaqGreen PCR Master Mix (2 X), Fermentas]. Amplification conditions were 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Amplified PCR products were purified and sequenced by TSINGKE Co. Ltd. (China).

Molecular phylogeny

The consensus sequence was generated from both ITS1 and ITS2 in BioEdit version 7.2.5 (Hall 1999) in the lab of Princes Nourah bint Abdulrahman University (Department of Biology, College of Science) and then searches were performed at the National Center for Biotechnology Information (NCBI) web site using BLASTn. The sequences generated during this study were submitted and deposited in GenBank (OM350446, OM350473). A dataset of 41 ITS based accessions were taken from published literature and downloaded from GenBank. Sequences were aligned and edited by using ClustalX 2.1 (Larkin & al. 2007) and BioEdit (Hall 1999). GenBank and newly generated sequences were aligned with MAFFT v. 10 (<http://mafft.cbrc.jp/alignment/server/index.html>, Katoh & Standley 2013). The alignment was manually edited and trimmed at 590 positions. These sequences representing 19 taxa were used to construct the phylogenetic tree. *Tomophagus colossus* was selected as outgroup (Zhou & al. 2015). MEGA 10.0 was used to run the maximum likelihood phylogenetic analysis with 1000 bootstrap replicates (Tamura & al. 2011).

Results

Molecular phylogeny

The phylogenetic tree produced by maximum likelihood analysis of cladistically informative sites is depicted in FIG. 1. The Pakistani *G. gibbosum* sequences (U2, U21) from our study made a well-supported clade with South Korean *G. gibbosum* together with *G. ellipsoideum* and *G. eickeri*; but less close to Colombian *G. gibbosum* (FIG. 1).

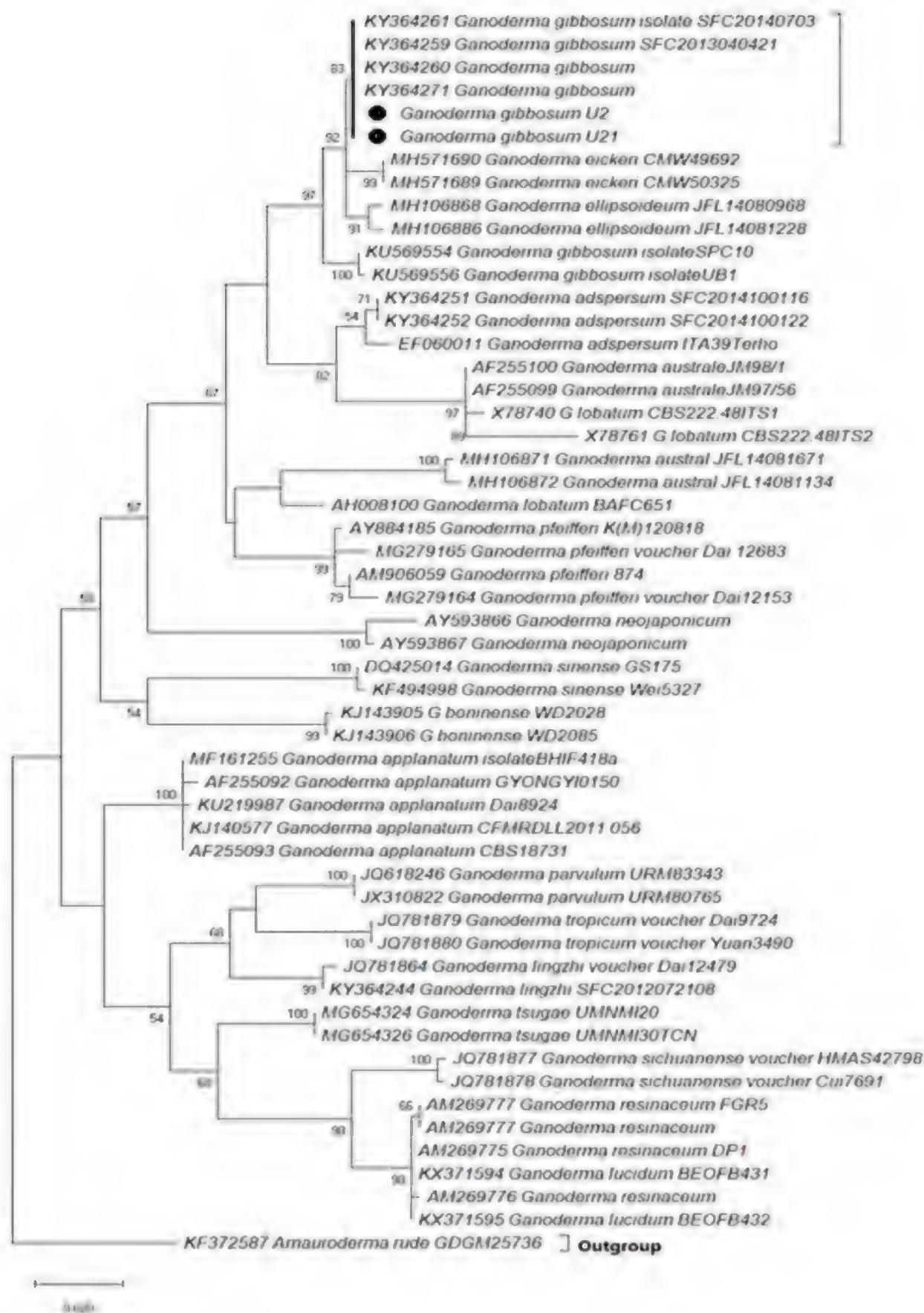


FIG. 1. Phylogenetic tree of *Ganoderma* based on ITS rDNA sequences generated by maximum likelihood, with *Amauroderma rude* as outgroup. Bootstrap values >50% are shown at the branches. Newly obtained sequences are marked with •.

Taxonomy

Ganoderma gibbosum (Blume & T. Nees) Pat., Ann. Jard. Bot. Buitenzorg, suppl. 1: 114 (1897) FIGS 2, 3

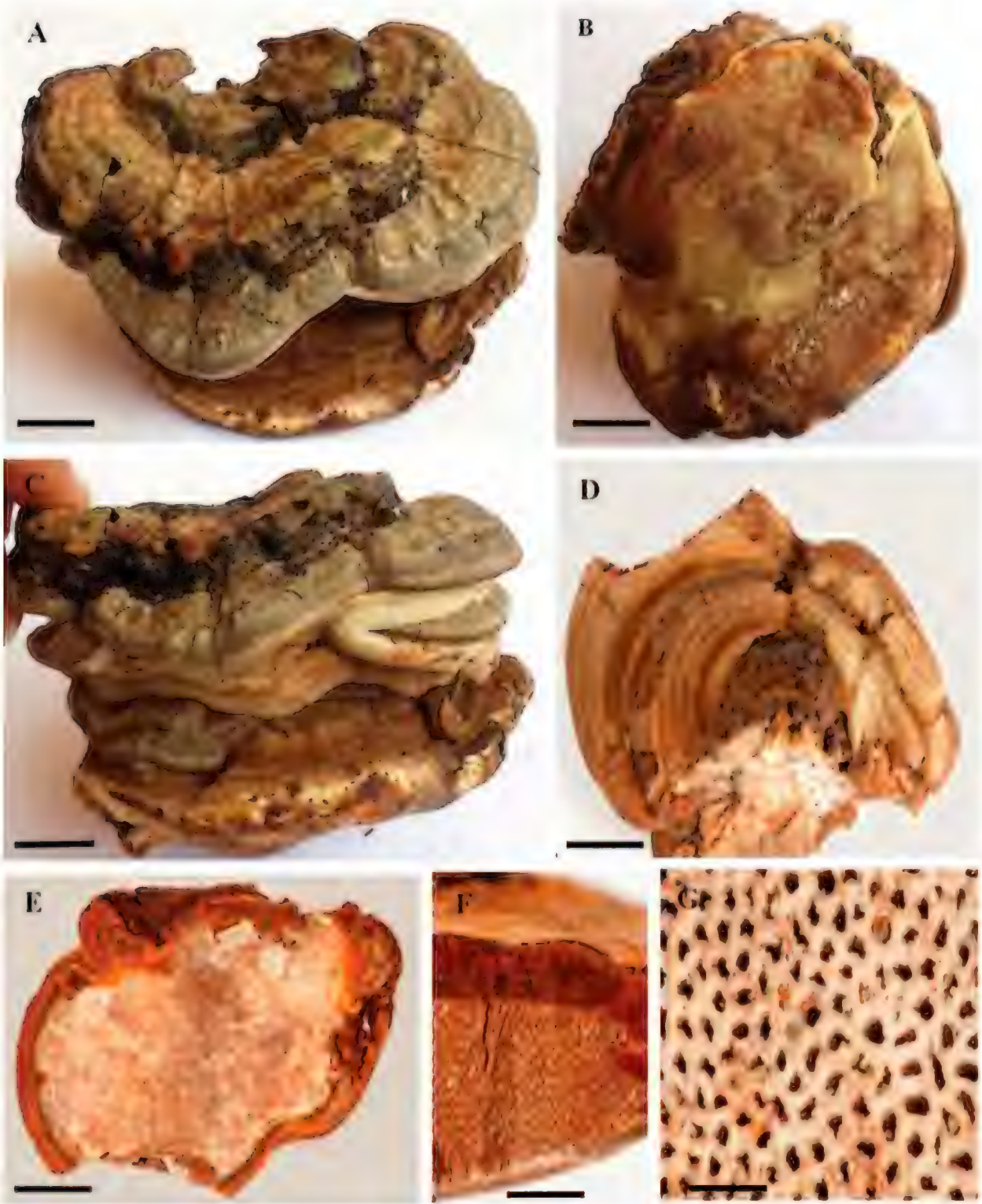


FIG. 2. *Ganoderma gibbosum* (TASM-ZSH091). A–C. Basidiomata; D, E. Pore surfaces and pores; F. Section of context and tubes. Scale bars: A–C = 2 cm; D, E = 2 mm; F = 1 mm.

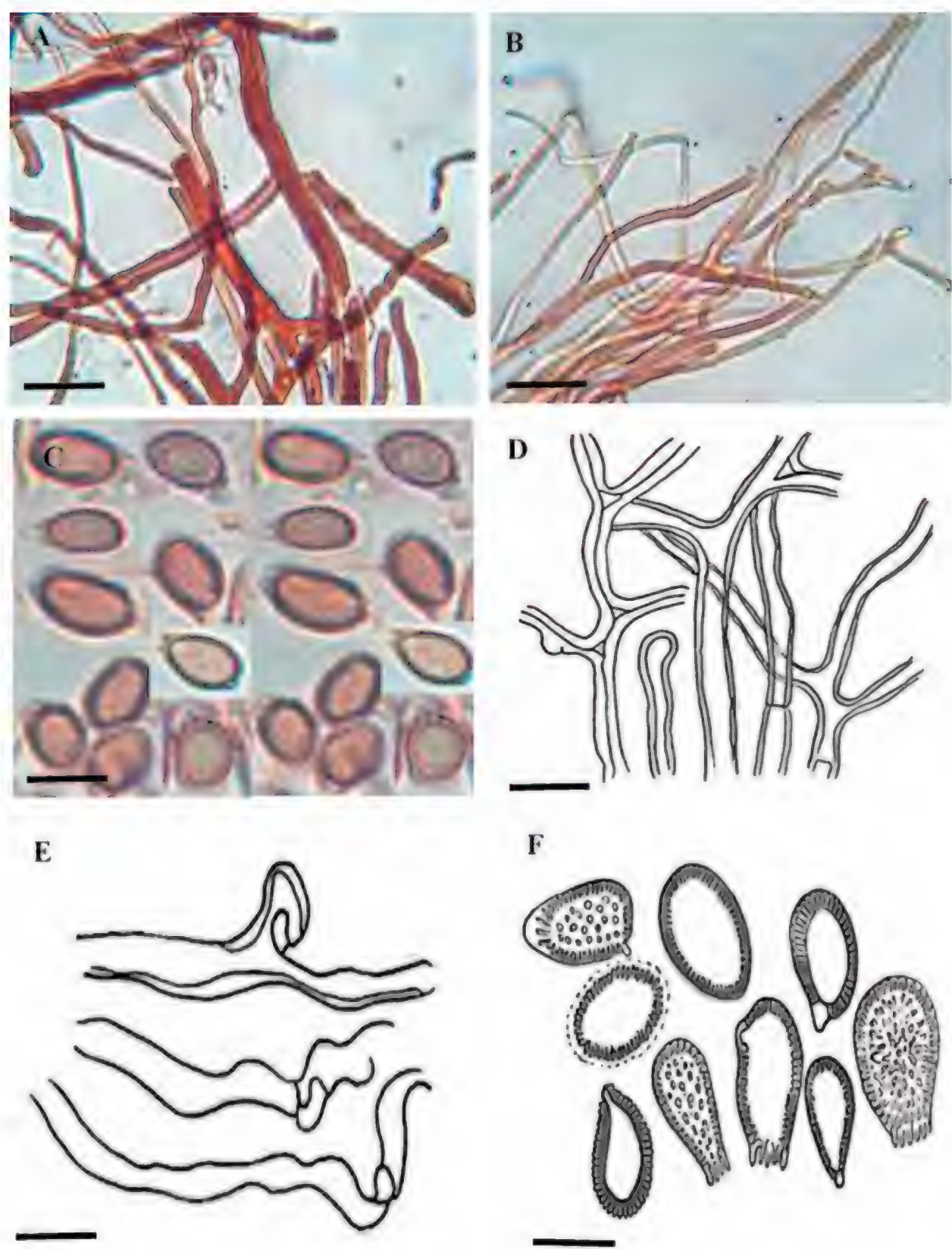


FIG. 3. *Ganoderma gibbosum* (TASM-ZSH091). A, B. Skeletal hyphae; C, D. Generative hyphae; E, F. Basidiospores. Scale bars: A–D = 5 μ m; E, F = 10 μ m.

BASIDIOMATA sessile, annual, woody, non laccate; PILEUS 9.0–9.4 × 4.5–4.8 cm, 4.5–4.8 cm thick, hard, sub orbicular, multilayered thick, whitish light brown to grayish brown, crust, concentric zones, turberculate bumps on surface, rivulose depressions, ridges; MARGIN several undulations, irregularities, 3 to 7 mm thick, concolorous to pileus; PORES 4–5/mm, light brown, sub circular to circular; TUBES 7–8 mm long, chocolate brown, grayish brown to golden; PILEIPELLIS/hymeniderm light brown to brown, branched cells, apically acanthus; HYPHAL SYSTEM dimitic: 1) skeletal hyphae 4.8–5.2 µm (width), brown, thick-walled, 2) generative hyphae 1.6–2.7 µm (width), hyaline, thin-walled; BASIDIOSPORES 7.5–10.2 × 4.6–5.9 µm, elongate to ellipsoid, light brown to brown, bitunicate eusporium, fine distinct short inter walled pillars, hyaline myxosporium.

SPECIMENS EXAMINED—**PAKISTAN, PUNJAB, Kasur district**, Changa Manga Forest, 31.0500°N 73.4072°E, 200 m a.s.l., gregarious on decayed hardwood, *Dalbergia sissoo* and *Acacia nilotica*, 15 July 2018, Aisha Umar, U21 (TASM-ZSH090; GenBank OM350473). **Lahore district**, Lahore, New Campus, University of the Punjab, 31.4981°N 73.3044°E, 217 m a.s.l., gregarious on hardwood, on living tree trunk of *A. nilotica*, 10 April 2019, Aisha Umar, U2 (TASM-ZSH091; GenBank OM350446).

Discussion

Ganoderma gibbosum is a non-laccate species reported from China, Korea, Vietnam, Laos, Thailand, Japan, Taiwan, India, Indonesia, Australia, USA, Mexico, Puerto Rico, Costa Rica, Colombia, Ecuador, Peru, Argentina, Brazil, and French Guyana (Blume & Nees von Esenbeck 1826; Saccardo 1888; Parmasto 1986; Moncalvo 2000; Cong 2010; Dai & al. 2011; Rojas & al. 2018; Hapuarachchi & al. 2018, 2019; Fryssouli & al. 2020). Dubious status and relationships in Neotropical *G. gibbosum* complexes is due to limited sequence data. Genetic approaches (Cabarro-Hernández & al. 2019; Tchoumi & al. 2018) provide valuable information to resolve phylogenetic patterns and mitigate limitations of orthodox methodologies (Fryssouli & al. 2020). Nevertheless, one of the possible hypotheses for parasitic and saprotrophic fungi on woody substrates, like *Ganoderma*, is that they may travel to long distances on introduced plants (Moncalvo 2000).

Our collections of *Ganoderma* from Pakistan agree with the description provided by Ryvarden (2000), and we report *G. gibbosum* as a new record based on molecular and morphological evidence. *Ganoderma gibbosum* from Pakistan was closely related to *G. gibbosum* and *G. eickeri* in the phylogeny inferred from the concatenated sequence data set. These species in *G. subg. Elfvingia* are easily distinguished by some macro- and microscopic characters of their basidiomes

(Tchoumi & al. 2018). Non-laccate species are heavily agglutinated and the massive matrix of pileal crust often obstructs the observation of discriminating features (Fryssouli & al. 2020).

Basidiomata of *Ganoderma gibbosum*, *G. ellipsoideum*, *G. adpersum*, *G. australe*, and *G. lobatum* [but not *G. eickeri*] are annual (Tchoumi & al. 2018; Hapuarachchi & al. 2019), sessile, non-laccate, woody, reniform, applanate to flabellate to dimidiate in shape, similar to our Pakistani specimens of our species. Basidiomata of our specimens are dark greyish to grey, while other non-laccate species range from reddish to brown in color. The pileus zones of *Ganoderma gibbosum*, *G. ellipsoideum*, *G. adpersum*, and *G. australe* are concentrically sulcate with tuberculate bumps and rivulose depressions, ruptured crust, margin concolourous with the pileus (Tchoumi & al. 2018; Hapuarachchi & al. 2019), like our Pakistani specimens.

The context of *G. eickeri* and *G. lobatum* (Tchoumi & al. 2018; Hapuarachchi & al. 2019) is soft, lightly woody, and homogenous chocolate brown, compared with dry, woody triplex in *G. ellipsoideum* and duplex in *G. gibbosum*, *G. adpersum*, and *G. australe* (Hapuarachchi & al. 2019), like our Pakistani specimens. Thickness of context is 4 mm to 12 mm in *G. lobatum* (Gottlieb & Wright 1999a), 3 cm in *G. eickeri* (Tchoumi & al. 2018), 1.5 cm in *G. gibbosum*, 2.5 cm in *G. ellipsoideum*, and 1.5 cm in *G. australe* (Hapuarachchi & al. 2019), whereas 6–9 mm in our Pakistani specimens. Basidiospore size of *G. adpersum* is $8.5\text{--}9.6\text{--}10.5 \times 5.4\text{--}6.1 \mu\text{m}$ (Hapuarachchi & al. 2019), *G. australe* $7.6\text{--}9.2\text{--}10.8 \times 5.3\text{--}7.6\text{--}7.9 \mu\text{m}$ (Bi & al. 1993; Hapuarachchi & al. 2019), *G. ellipsoideum* $6.1\text{--}7.3 \times 3.7\text{--}4.6 \mu\text{m}$, *G. eickeri* $8.5\text{--}11 \times 5\text{--}7 \mu\text{m}$ (Tchoumi & al. 2018), *G. lobatum* $7.5\text{--}11 \times 5\text{--}7 \mu\text{m}$ (Steyaert 1980), and *G. gibbosum* $6.9\text{--}7.6 \times 4.6\text{--}5.6 \mu\text{m}$ (Hapuarachchi & al. 2019), all similar to our specimens ($7.5\text{--}10.2 \times 4.6\text{--}5.9 \mu\text{m}$). These are ranged from ovoid to ellipsoid, bearing fine, short and distinct inter walled pillars with overlaid hyaline myxosporium. Basidiospores disseminate by wind and human activities (Gonthier & al. 2004; Moncalvo & Buchanan 2008), which may facilitate the broad geographic distribution of *Ganoderma* species, especially those present in more than one locality (Gonthier & al. 2004; Moncalvo & Buchanan 2008; Tchoumi & al. 2018).

On angiosperms, *Ganoderma eickeri* originates from South Africa (Tchoumi & al. 2018). However, the ITS data of *G. eickeri* are closely related to *G. gibbosum*. Sequences of *G. ellipsoideum* subclade were mainly labelled as “*G. gibbosum*”, “*G. australe*”, and “*G. applanatum*”, and originated from broad geographic distribution (south and east Asia, Oceania) on both eudicots and monocots, but not on gymnosperms (Fryssouli & al. 2020).

We conclude that morphological plasticity and substantial overlapping of “diagnostic” characters are prevalent in these ‘dull’ taxa, so that their taxonomic importance is dubious. This suggested that laccate or non laccate traits can be used for demarcation of *Ganoderma* species at the subgeneric level, but do not always reveal the true phylogenetic relationships.

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